International Journal of Medicine and Pharmaceutical Sciences (IJMPS)
ISSN(P): 2250-0049; ISSN(E): 2321-0095
Vol. 4, Issue 4, Aug 2014, 47-54

EXTRACTION AND PURIFICATION OF β -GALACTOSIDASE FROM A LOCAL ISOLATE OF *LACTOBACILLUS ACIDOPHILUS*

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ABSTRACT

The aim of the present study was conducted to study the purification of β-galactosidase isolated from local isolate *Lactobacillus acidophilus* have been used in the dairy industry for the improvement of lactose intolerance. The β-galactosidase produced *Lactobacillus acidophilus* was extracted and purified by precipitation with ammonium sulphate and dialysis followed by gel filtration using sephadex G-200 chromatography. The result found the ammonium sulfate (80%) was selected as the best ratio for the precipitate of lactase enzyme. These purification steps raised the specific activity to (0.00000108) U/mg in precipitation step with purification folds (4.7) and yield (73%). The specific activity increased in dialysis step to (0.00000154) U/mg with purification fold (6.7) and yield (55.7%). In gel filtration step, the specific activity increased to (0.0000075) U/mg with purification fold (6.9) and yield (33.9%). The molecular weight of purified lactase approximately 93 KDa using the SDS-PAGE.

KEYWORDS: β-galactosidase, *Lactobacillus acidophilus*, Lactic Acid Bacteria, Lactose Digestion

INTRODUCTION

Lactase (β-D-galactosidase EC 3.2.1.23) is a hydrolytic enzyme that has two enzymatic activities; one is responsible for the hydrolysis of lactose and cleaves cellotetrose and cellotriose and the second activity associated with splitting β-glycosides [1]. β -galactosidase hydrolyzes lactose into glucose and galactose, which can be readily absorbed and hence caters to lactose intolerant people [2]. Lactose intolerance is a problem for people who have a low amount of intestinal β-galactosidase activity and for whom lactose behave like non-digestible carbohydrate [3]. Approximately two-third people of the world are accounted to having the problem of lactose intolerance affecting their quality of life[4].

The symptoms of lactose intolerance include abdominal pain, diarrhea, nausea, and bloating after the ingestion of lactose or lactose containing food substances. Treatment is relatively simple by eliminating lactose from the diet or by using of supplemental β- galactosidase enzyme replacement [5].

Lactase is important commercial enzyme having several applications in the food, pharmaceutical industries and have a significant role in texture and flavor of food products [6-7]. Commercial β-galactosidase is produced from microorganism including bacteria, yeast, and mold [8]. Among bacterial sources, *Lactobacillus acidophilus* [9], *Streptococcus pneumonia* [10] and *Bifidobacterium adolescentis* [11] *Lactobacillus acidophilus* naturally produces several compounds that are beneficial to the human body, including: vitamin K, the enzyme lactase, and the anti-microbial substances such as acidolin, acidolphilin, lactocidin, and bacteriocin [12]. Therefore, the aim of present study was the isolation, extraction, and purification the β-galactosidase enzyme from local isolate of *Lactobacillus acidophilus*.

MATERIALS AND METHODS

This study has been conducted between November 2013 to March 2014, at the laboratories of the Biology Department, Babylon University.

Bacterial Strains and Growth Conditions

Four *Lactobacillus acidophilus* were grown in MRS broth at 37°C for 24 hours, and were characterized as previously described [13].

Detection of Lactase Produced by *Lactobacillus acidophilus*

The ability of *Lactobacillus acidophilus* to produce lactase enzyme was investigated for four isolates (Lac1, Lac2, Lac3 and Lac4) using O-nitrophenyl galacto pyranosid (ONPG) as substrate.

Preparation of O-Nitrphenol Standard Curve

The activity of β- Galactosidase was measured using a standard curve of different concentrations of O-nitrophenol (ONP) was prepared as describe by Noah and Gilliland [14]. Optical densities were measured spectrophotometrically at 420nm for each concentration.

Enzyme Extraction

The β- Galactosidase preparation was performed according to the method of [15]. Briefly, all *Lactobacillus* isolates were grown in MRS broth (100 ml). Cells were harvested by centrifugation at 12.000 x g rpm for 20 minutes at 4 °C. The cell free supernatants were kept under freezing until used.

Measurement of β- Galactosidase Activity

An assay of β-galactosidase activity was carried out using ONPG as the substrate as described by Atlas *et al* [16]. Tubes contacting 0.5 ml of culture and 0.5 ml of Z-buffer were placed in a water bath at 30 C for 3 min. Cells were lysed by adding 25 µl of 1% SDS, 50 µl of chlorofom and vortex for 20 second, then 200 µl of ONPG (4mg/ml) was added and the tubes were incubated at 30 °C for 30 min. 2 ml of Na_2CO_3 (1M) was added and tubes were transferred to ice bath. This mixture was centrifuged at 5000 rpm for 10 min to remove cell debris. Supernatants were taken and the optical density was recorded by spectrophotometer at 420 nm.

One unit of enzyme activity (U) is defined as 1µmol of ONPG formed per ml per min under the assay conditions. Enzyme activity was determined by using the ONPG standard curve applying the following equation: µmol of liberated ONPG / T * V, While T = time of reaction and V= volume of culture.

Determination of Protein Concentration

Protein concentration was determined by using the Bradford method [17] using Serum Albumin (BSA) as a protein standard. The optical density of the reaction mixture was measured spectrophotometrically at 595 nm.

Purification of β-galactosidase Enzyme

β -galactosidase enzyme was purified from *lactobacillus* using techniques that included precipitation with ammonium sulfate, dialysis, gel filtration and SDS-PAGE to deduce the purity of the enzyme

- **Precipitation with Ammonium Sulfate**

The ammonium sulfate was used at different saturation ratios (60, 70, 80 and 90 %), to identify the optimal ratio for precipitation the enzyme. The salt was added gradually to 10ml of enzyme solution in ice bath under stirring. Mixtures were centrifuged at 4000 rpm for 20 min. The supernatant was discarded while the precipitate was dissolved in a minimum volume of phosphate buffer. Enzyme activity and protein concentration were then measured.

- **Dialysis**

The ammonium sulfate precipitate (in solution) was dialyzed using a dialysis bag (cutoff 100KDa) overnight at 4 °C against phosphate buffer (pH 7). The obtained enzyme preparation was concentrated against crystals of sucrose and kept in the refrigerator at 4 °C for further purification.

- **Enzyme Separation through Gel Filtration Chromatography**

The enzyme solution produced from dialysis (6ml) was added gently on the surface of the sephadex G-200 column. The elution was achieved by using phosphate buffer (pH 7) at flow rate 0.2 ml / 1 min. Three milliliters were collected per each fraction. The protein concentration in the fractions was measured at 280 nm and the specific activity was calculated after measurement of the enzymatic activity.

- **Protein Analysis and Gel Electrophoresis**

Protein concentrations were determined by the method of Bradford [17] using bovine serum albumin as the standard. The purity and molecular weight of purified enzyme was determined by SDS-PAGE (12.5%). The electrophoresis was carried at 100 volts for 4h. Protein bands were visualized by staining with Coomassie brilliant blue R-250 [18].

RESULTS & DISCUSSIONS

Lactobacilli are extensively used for fermentation of dairy products, fish and meat, therefore, have been investigated intensively for their industrial applications [19].The best sources for commercial β-galactosidase enzyme for the commercial production and treatment of lactose intolerance, are microbial in nature such as *Aspergillus niger*, *Lactobacillus spp* and *E. coli* [8].

In the present study, four *lactobacillus acidophilus* (Lac1, Lac2, Lac3 and Lac4) were screened for the production of lactase enzyme. All the strains were found to be lactose producing as shown in table 1, but the strain that has the highest activity (Lac4) was selected for this study. After choosing the best strain for production lactase, the enzyme was purified using precipitation by ammonium sulfate, dialysis, gel filtration chromatography, and polyacrylamide gel electrophoresis.

Table 1: Enzyme Activity and Protein Concentration for *Lactobacillus acidophilus*

Bacterial Strain	Enzyme Activity (U/ml)	Protein Concentration (μg/ml)
Lac1	0.000485	55.37
Lac2	0.000007	50.51
Lac3	0.0001	48.62
Lac4	0.000885	56.45

- **Precipitation with Ammonium Sulfate**

In order to concentrate the crude extract of β-galactosidase enzyme and remove a much of water and some protein molecules as possible, the ammonium sulfate concentrations used for different saturation ratios are shown in Table 2. It was found that 80% saturation was the best ratio for precipitation the crude extract for enzyme.

Table 2: Ratios of Ammonium Sulfate Precipitation of Lactase Enzyme

% of Ammonium Sulfate Precipitation	Enzyme Activity U/mg
60%	0
70%	0.0003
80%	0.0012
90%	0.0003

Protein precipitation by ammonium sulphate depends on the salting out phenomenon. Since ammonium sulphate has the ability to neutralize charges at the surface of the protein and to disrupt the water layer surrounding the protein, it will eventually cause a decrease in the solubility of the protein which, in turn will lead to the precipitation of the protein by the effect of salt [20].

Specific activity reached to (0.00000108) U/mg with purification fold (4.7) and yield (73%) as shown in table 3. Mozumder *et al* [15] reported extraction this enzyme from *lactobacillus* isolated from yogurt by precipitation with 80% ammonium sulfate.

The result of this study agrees with the reported findings. Sumathy *et al* [21] used the cold acetone for precipitation the enzyme produced from *lactobacillus spp*. isolated from milk products. The ammonium sulfate was used in enzyme precipitation because of its high solubility, low cost compared with other salts, and did not pH and enzyme stability.

Table 3: Purification Steps of *L. acidophilus* B-galactosidase Enzyme

Purification Step	Enzyme Activity (U/l)	Protein Concentration (µg/l)	Specific Activity (U/µg)	Total Activity (U)	Purification Folds	Yield (%)
Crude extract	0.00017407	75.8888	2.2938E-06	0.005222	1	100
Ammonium sulfate precipitation 80%	0.00038148	35.0808	1.08744E-05	0.003814	4.74076003	73.04
Dialysis	0.00048518	31.3434	1.54796E-05	0.002911	6.74846579	55.74
Gel filtration chromatography (sephadex G-200)	0.00064814	8.616	7.52247E-05	0.001296	6.917618	33.98

- **Dialysis**

After ammonium sulfate precipitation, the enzyme was dialyzed overnight against phosphate buffer, the specific activity reached to (0.00000154) U/mg with a purification fold of 6.7 and yield of 55.7% as shown in Table 3. A study by Mozumder *et al* [15] used dialysis against 10 mM Tris-HCl (pH 7) for 24 hours.

- **Gel Filtration Chromatography**

A Kolker *et al* [22] reported the purification of β-galactosidase from *L.acidophilus* using HIC column. DEAE-cellulose chromatography was also used for the purification of the enzyme. In this study, the enzyme purified using gel filtration on sephadex G-200 column (15*1 cm). Development of the column produced one peak of purified enzyme as shown in figure 1. The specific activity of the enzymes from the harvested peak was (0.0000075) U/mg with a purification fold of 6.9 and a yield of33.9% as shown in Table 3. Similar results were reported by AKolker *et al* [22] who purified the enzyme from fermented ragi using sephadex G-200.

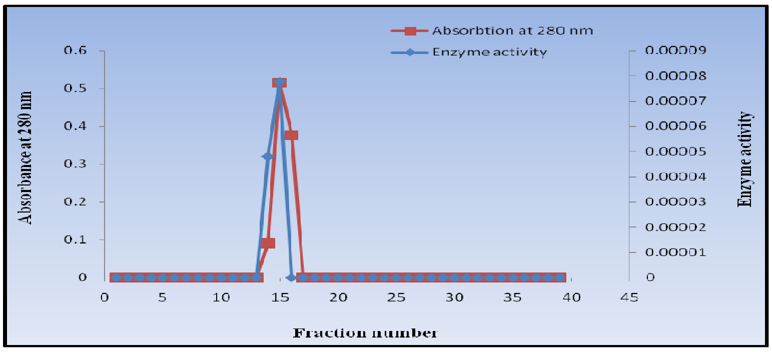

Figure 1: Gel Filtration Chromatography Using DEAE-Cellulose Sephadex G-200 Column (15×1) Cm with Phosphate Buffer (Ph 7), Flow Rate12 Ml/Hr and Fraction Volume 3ml for Purification Lactase

- **Gel Electrophoresis of Lactase**

In order to investigate the purity of the lactase purified from Lactobacillus (Lac4), polyacrylamide gel electrophoresis under denaturing conditions was used. There was one band with molecular weight 93 KDa (Figure 2). Other molecular weight studies for β-galactosidase on SDS-PAGE reported are: 110 KDa in *lactobacillus acidophilus* [21], 45KDa in *lactobacillus acidophilus* PTCC 1643 [23] and 518KDa in *E. coli* [24]. Pastore and Park [25] purified β-galactosidase from *Scopulariopsis spp* by precipitation with ammonium sulphate and two chromatographic steps leading to a 4% yield of the pure enzyme desired.

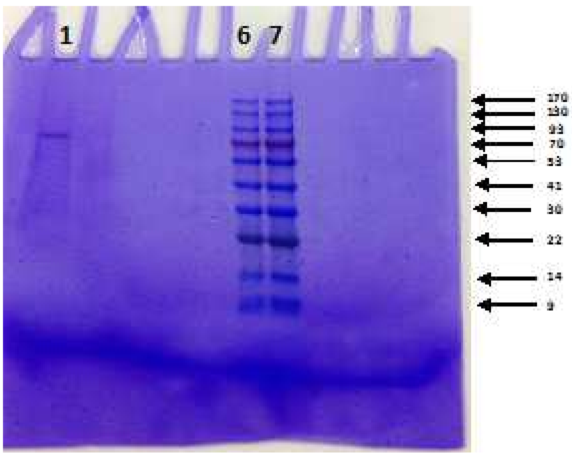

Figure 2: SDS-PAGE Electrophoresis of the Lactase Enzyme from Lactobacillus under Denaturing Conditions Lane 1: Represents the Purified Enzyme, Lanes 6 &7: Protein Ladder

REFERENCES

1. Sheik, A. S. and P. Gunasekaran. (2010). Corrent trends of beta galactosidase research and application. Current Research, Technology Education Topics in Applied Microbiology and Microbial Biotechnology. Formatex..A.Mendez-Vilas (Ed).pp 880-890.
2. Paige,D.M.(2005). Lactose intolerance, in Encylopedia of human nutrition, 2 nd, (Elsevier Ltd, Oxford).113-120.
3. Karasova, P.; Spiwok, V.; Mala, S.; Kralova, B. and Russell, N. (2002). Beta-Galactosidase Activity in Psychrotrophic Microorganisms and their Potential Use in Food Industry. Czech J. Food Sci. 20: 43.
4. Pribila, B. A.; Hertzler,S. R and Martin, B.R.(2000). Improved Lactose Digestion and Intolerance. Journal of the American Dietetic Association. J. Am. Diet. Asso. 100(5): 524.
5. Vasiljevic, T and Jelen P. (2001). Production of β-galactosidase for lactose hydrolysis in milk and products using thermophilic lactic acid bacteria. J. Innov. Food Sci. Emerg. technol. 2: 75-85.
6. Bhowmick, T.; Johnson, M.C. and Bibek, R. (1987). Factors influencing synthesis and activity of β -galactosidase in *Lactobacillus acidophilus*. Journal of Industrial Microbiology 2: 1–7.
7. Chammas G.I, Saliba R., Corrieu G., and Beal, C. (2006). Characterization of lactic acid bacteria isolated from fermented milk. Int. J. Food Microbiol. 110: 52-61.
8. Panesar, P.S.; Panesar, R.; Singh, R.S.; Kennedy, J.F. and Kumar, H. (2006). Microbial production, immobilization and applications of β-D-galactosidase. Journal of Chemistry Technical Biotechnology 81: 530-543.
9. Nguyen, T. H.; Splechtina, B.; Steinböck, M.; Kneifel, W.; Lettner, H. P.; Kulbe, K. D. and Haltrich, D. (2006). Purification and characterization of two novel β-galcactosidases from *Lactobacillus reuteri*. J. Agric. Food Chem. 54, 4989-4998.
10. Jeong**,** J. K.; Kwon, O. S.; Lee, Y. M.; Oh, D. B.; Lee, J. M.; Kim, S. H.; Kim, E. H.; Lee, T. N.; Rhee, D. K. and Kang, H. A. (2009).Characterization of the Streptococcus pneumonia BgaC protein as a novel surface β-galactosidase with specific hydrolysis activity for the Gal β1-3GlcNAc moiety of oligosaccharides. J. Bacteriol. 191, 3011-3023.
11. Hinz**,** S. W. A.; van den Broek, L. A. M.; Beldman, G.; Vincken, J. P. and Voragen, A. G. J. (2004). β-Galactosidase from *Bifidobacteriumadolescentis* DSM290083 prefers β(1,4)- galactosides over lactose. Appl. Microbiol. Biotechnol. 66: 276-284.
12. Ljungh**,** A. and Wadström, T. (2006).Lactic acid bacteria as probiotics. *Current Issues in Intestinal Microbiology.* 7: 73-89.
13. Abdulla AA, Jebor MA, Abed TA. (2013). Isolation and identification by PCR analysis for probiotic properties of *Lactobacillus* sp from dairy products. J. Biol. Agri. Heathcare. 3:83-89.
14. Noah, D. O.; Gilliland, S. E. (1994). Influence of bile on β- galactosidase activity of component species of yogurt starter cultures. *J.Dairy Sci.*, Champaing, v. 77, p. 3522-3537.

15. Mozumder, N. H..; Akhtaruzzaman, M.; Bakr, M.A. and Fatema-Tuj-Zohra (.2012).Study on isolation and partial purification of lactose (β-galactosidase) enzyme from Lactobacillus bacteria isolated from yogurt. J.Sci.Res. 4(1):239-249.

16. Atlas, M.; Parks, C. and Brown, A. (1995). Laboratory Manual of Experimental Microbiology. Mosby – Year – Book, Inc., USA.

17. Bradford**,** M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry.72:248-254.

18. Laemmli, U.K. (1970). Cleavage of structure proteins during the assembly of the head of bacteriophage T4. Nature. 277: 680-685.

19. Konings**,** W. N.; Kok, J.; Kuipers, O. P.; Poolman, B. (2000). Lactic acid bacteria: the bugs of the new millennium. Curr. Opin. Microbiol., 3: 276-282.

20. Englard**,** S. and Seifter, S. (1990). Precipitation Techniques. In: Murray, E.D. and P. Dentscher (Eds.), Methods in Enzymology.182: 425-441.

21. Sumathy, R.; Vijayalakshmi, M. and Deecaraman, M. (2012). A study of β-galactosidase of *Lactobacillus*sp from milk products and its application. International Journal of Applied Biology and Pharmaceutical Technology, 3(4): 138-148.

22. Akolkar, S.K., A. Sajgure and S.S. Lele, (2006). β-galactosidase production from *Lactobacillus acidophilus* isolated from fungi ragi. *Indian Journal of Biotechnology*, 5: 184-188.

23. Farzadi**,** M.; Khatami, S.; Mousavi, M. and Amirmozafari. N. (2011). Purification and characterization of β –galactosidase from *Lactobacillus acidofillus*. African Journal of Biotechnology Vol. 10(10): 1873-1879.

24. Sund, H. and Weber, K.(1963). Milk sugar cleaving enzyme (XIII), size and content of β- galactosidase from E. coli.Biochemistry.24:337-340.

25. Pastore**,** G.M and Park, Y.K. (1981). Beta-D-galactosidase from Scopulariopsis sp.: immobilization on phenol-formaldehyde (Duolite) resin, and efficiency for hydrolysis of lactose in whey**.** *Cienc. Technol. Aliment.*1:65.